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Vaccination of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth performance[1]

F. R. Dunshea*[2], C. Colantoni†, K. Howard†, I. McCauley*, P. Jackson*, K. A. Long*, S. Lopaticki*, E. A. Nugent*, J. A. Simons*, J. Walker†, and D. P. Hennessy†

*Agriculture Victoria, Victorian Institute of Animal Science, Werribee, VIC 3030, and Attwood, VIC 3049 Australia and †CSL Ltd, Poplar Road, Parkville, VIC 3052, Australia

ABSTRACT: Peri- and postpubertal boars accumulate substances (e.g., androstenone and skatole) in their fatty tissue that are responsible for boar taint in pork. The objective of this study was to assess the efficacy of a GnRH vaccine, Improvac, in eliminating boar taint. Three hundred male (200 intact boars, 100 barrows) crossbred (Large White × Landrace) pigs were used in a 2 × 3 factorially arranged experiment. The respective factors were sex group (barrows, boars treated with placebo, or boars treated with Improvac) and slaughter age (23 or 26 wk). Vaccines were administered 8 and 4 wk before slaughter. All Improvac-treated pigs exhibited anti-GnRH titers. Testes and bulbo-urethral gland weights in treated pigs were reduced by approximately 50% ($P < 0.001$) and serum testosterone levels were below 2 ng/mL in the majority of treated boars (94 and 92% across both age groups at 2 and 4 wk, respectively). Boar taint, as assessed by the concentration of androstenone and skatole in s.c. fat, was suppressed to low or undetectable levels in 100% of Improvac-treated boars. No Improvac-treated pigs had significant concentrations of either androstenone (> 1.0 μg/g) or skatole (> 0.20 μg/g). In contrast, 49.5% of placebo-treated controls had significant androstenone and 10.8% had significant skatole levels, resulting in 10% of the control boars with high concentrations of both compounds. The mean concentrations of taint compounds in the Improvac-treated pigs were not significantly different from those in barrows. Improvac-treated boars grew more rapidly ($P = 0.051$ and < 0.001 for pigs slaughtered at 23 and 26 wk of age, respectively) than control boars over the 4 wk after the secondary vaccination, possibly because of reduced sexual and aggressive activities. Compared with barrows, Improvac-treated boars were leaner and had superior feed conversion efficiency. The vaccine was well tolerated by the pigs, and no observable site reactions could be detected at the time of slaughter. Vaccination of boars with Improvac allows production of heavy boars with improved meat quality through prevention and control of boar taint.

Key Words: Boar Taint, GnRH, Growth, Pigs, Skatole, Vaccines

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Introduction

During sexual development and when mature, boars accumulate substances, predominantly androstenone and skatole, in their fatty tissue that are regarded as the main contributors to boar taint in pork (Bonneau, 1982). To avoid tainting of the meat, boars destined for fresh meat consumption in Australia and New Zealand have, until recent years, been slaughtered before sexual maturity. In other countries taint is overcome by castration of the boar before weaning. However, castration results in significant reductions in growth performance and excess deposition of fat (Campbell and Taverner, 1988; Dunshea et al. 1993b).

Over recent years, the average weight of pigs at slaughter in Australia has increased (Pig Stats, 1998), driven by the efficiencies associated with the slaughter of heavier pigs. Because boar taint increases with sexual maturity, the increase in slaughter weight has been associated with an increase in the risk of boar taint. One method of inhibiting sexual development and boar taint is immunization against GnRH (Caraty and Bonneau, 1986; Dunshea et al. 1993a; Bonneau et al., 1994). However, most vaccine regimens reported to date have been inappropriate because they have required many injections or the site reactions that

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[2]Correspondence: Private Bag 7, Sneydes Road (Phone: 61 3 9 742 0438; fax: 61 3 9 742 0400; E-mail: Frank.Dunshea@nre.vic.gov.au).

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occurred after injection of adjuvants required a long vaccination-to-slaughter interval.

Recently, a vaccine (Improvac) has been developed that contains a modified form of GnRH in an aqueous adjuvant system that causes little tissue aggravation. The vaccine formulation and protocol allows the pigs to receive the secondary vaccine around 4 to 5 wk before slaughter.

Any taint substances already present are progressively metabolized, allowing the entire boar to be slaughtered at a heavier BW without taint and after having benefited from the effects of its own testicular steroids on growth. The objectives of this study were to assess the efficacy of Improvac in suppressing boar taint and improving growth performance in boars.

Materials and Methods

Animals and Handling. All procedures involving animals were approved by the Victorian Institute of Animal Science Animal Ethics Committee and the study was conducted at a commercial pig enterprise. Three hundred male (200 boars and 100 barrows) crossbred (Large White × Landrace) pigs were used in a 2 × 3 factorially arranged experiment. The respective factors were sex group (barrows, boars treated with excipient or boars treated with Improvac) and slaughter age (23 or 26 wk). Pigs were from contemporary farrowings and pigs destined for the barrow group were castrated between 1 and 2 wk of age. Pigs were weighed and selected at 15 wk of age and, after stratification on live weight, were allocated within sex to pens of 20 pigs. For each pen of 20 intact male pigs, 10 were vaccinated with the first dose of Improvac and 10 with placebo vaccine, following a predetermined schedule compiled by random number generation. As each pen of 20 pigs was moved to the finisher shed, at 16 wk of age, the pigs were split into pens of 10 according to treatment. Pigs were fed commercial, unmedicated, wheat-based grower or finisher diets for ad libitum consumption. The grower and finisher diets also contained 20 and 12% lupin kernels, respectively. The diet specifications were 14.0 and 13.3 MJ DE and 20.1 and 16.4% CP for the grower (15 to 19 wk of age) and finisher (beyond 19 wk of age) diets, respectively. Pigs were weighed and feed intake was measured on a per-pen basis weekly from 19 wk of age. Pigs to be slaughtered at 23 wk of age were vaccinated at 15 and 19 wk of age, whereas pigs to be slaughtered at 26 wk of age were vaccinated at 18 and 22 wk of age. Boars were vaccinated under a double-blind study protocol with either Improvac or a placebo vaccine (2 mL), which were manufactured and formulated by CSL Ltd. (Parkville, Victoria, Australia). Prior to use the vaccine was dispensed into group-coded 2-mL disposable syringes. Pigs were bled by jugular venipuncture and testes width measured at the commencement of the experiment at 15 wk of age. Those pigs given the primary vaccination at 15 wk of age were bled and their testes width was measured again at 19, 21, and 23 wk of age. Those pigs given the primary vaccination at 18 wk were bled and their testes width was measured at 18, 22, 24, and 26 wk. Testes size was assessed by measuring the maximum total scrotal width using a set of engineering calipers.

The injection sites of each pig were inspected by physical palpation of the area surrounding the injection at weekly intervals for 4 wk following administration of each dose of vaccine. Reactions were scored according to the following system: 0, no reaction detected; 1, reaction detected by palpation only and not visible or reddened; 2, reaction visible as a swelling or lump, but no evidence of abscessing; 3, reaction considered an unruptured abscess; and 4, reaction abscessed and ruptured.

At slaughter, severe "fighting lesions" around the head and shoulders were recorded. Pigs were described as either having or not having fighting lesions, defined as scratches and bite marks, that probably arose due to fighting during transport to the abattoir or in the overnight lairage at the abattoir prior to slaughter. Testes and bulbo-urethral glands were removed from the carcass. Testes were trimmed and weighed. Bulbo-urethral glands were trimmed of all connective tissue and weighed and their length was measured. Carcass weight and backfat at the P2 point (6.5 cm from the midline over the last rib) were measured on line. Belly fat samples were removed from the carcasses after cooling.

Chemical Analyses. Testosterone concentration in serum was measured using a commercial radioimmunoassay kit (Orion Diagnostica, Espoo, Finland). The method followed the manufacturer's recommended procedure with the following modifications. A 100-μL aliquot of standard, control, or unknown was extracted by vortex mixing for 15 min with 1 mL of a 9:1 (vol/vol) diethyl acetate/ethyl acetate mixture. A 100-μL aliquot of the organic extract was then removed and dried under nitrogen. The remainder of the assay was performed as per kit instructions with the exception that the volumes of antibody, tracer, and second antibody were all reduced by 50%. The limits of quantification of the assay were 0.5 to 100 ng/mL. Intra- and interassay variation for low, medium, and high control samples were 10, 10, and 7%, and 12, 11, and 10%, respectively.

Androstenone and skatole were measured by the method of Hansen-Møller (1994) with the following modifications. Samples were rendered by microwave and 200-μL samples were extracted by vortex mixing with 1 mL of 95:5 (vol:vol) methanol/water containing 0.25 μg/mL androstenone and 0.05 μg/mL 2-methyl indole as internal standards. The extract was centrifuged at 2,000 × g for 10 min at 0°C, and the supernate was placed in sample vials. Samples were derivatized according to the method of Hansen-Møller (1994) except that boron trifluoride and dansyl hydrazine were premixed before adding 12 to 20 μL of the extracted

sample. Chromatography was conducted using a Waters 2690 Separations module (Waters Co., Milford, MA), on a 4- × 100-mm, 3-μm particle size Hypersil ODS column (Hewlett Packard, Blackburn, Australia) and peaks were detected by a Waters 474 fluorescence detector. The limits of quantification of the assay were 0.2 to 2.4 μg/g and 0.03 to 0.6 μg/g for androstenone and skatole, respectively. Intraassay variation for two samples with low and high concentrations were 5 and 2% for skatole and 6 and 8% for androstenone, respectively. Interassay variation for two samples with low and high concentrations were 5 and 7% for skatole and 10 and 12% for androstenone, respectively.

Antibodies to GnRH were measured by noncompetitive RIA. Duplicate serial dilutions of serum in phosphate buffered saline (**PBSGA**) (0.1 mol/L sodium phosphate buffer, pH 7.4, 0.09% NaCl, 0.1% gelatin, 0.1% NaN_3) of 200 μL, covering the range from 1/200 to 1/3,200, were incubated with 100 μL of PBSGA containing 17,500 dpm of [^{3}H]GnRH (New England Nuclear, Boston, MA) and 100 μL of 5 mg/mL human gamma globulin (Normal Immunoglobulin, CSL Ltd, Parkville, Australia) for 48 h at 4°C. Tubes containing buffer instead of serum were prepared for measuring the nonspecific binding (**NSB**). At the end of incubation, the labeled GnRH bound by antibodies was precipitated by adding 100 μL of 5% bovine gamma globulin (Pentex, Bayer, Kankakee, IL) in PBSGA and 1 mL of saline containing 18 g/100 mL of polyethylene glycol 6000 (BDH, Poole, U.K.).

After mixing, the tubes were centrifuged at 2,000 × *g* for 10 min and the supernate was decanted. The pellet was redissolved by mixing with 500 μL of 0.1 mol/L NaOH and 4 mL of scintillant was added (Optiphase Hisafe 2, Wallac, Turku, Finland) and counted on a Wallac 1410 counter. Total counts (**TC**) were determined from tubes to which only the labeled GnRH had been added. After subtraction of counts bound nonspecifically, the binding in each tube (**B**) was expressed as the fraction of the total counts bound by each dilution (%B/TC). For each sample, a binding curve of the logit of (%B/TC) against the log of the reciprocal of the dilution was analyzed by linear regression and the reciprocal of the dilution that was equivalent to 30% binding was designated as the titer. The limits of quantification of the assay were titers from 20 to 3,500. Intra and interassay variation for low and high control samples were 5 and 7% and 12.5 and 13.9%, respectively.

Statistical Analyses. Although the study was a 2 × 3 factorial design, to avoid confounding of age and time the data were analyzed as two separate experiments. For all continuous data, such as testes width, live weight, and plasma testosterone concentration, analysis of variance was used. Analytical data above the limit of detection were treated as if equal to the upper limit. Data below the limit of quantification were treated as halfway between the limit and zero. In each case the assumptions of analysis of variance were assessed graphically by use of residual plots. In the cases in which the assumptions were deemed to be met, the group means, the least significant difference (LSD), and the *P*-value were tabulated. In cases in which the assumptions did not hold, a suitable transformation was attempted. The transformations used, in order or preference, were square root, natural log, or double natural log. If the assumptions were deemed valid after transformation, the inferences (significance/nonsignificance) were examined to determine whether the transformation had altered these inferences. Because in all cases the inferences had not changed, the results obtained from the raw data have been presented as outlined above. In cases in which the assumptions were deemed to have not held and a suitable transformation could not be found, the Kruskal-Wallis nonparametric analysis of variance was used. In such circumstances group means and group medians are presented along with the range of the data in the case of anti-GnRH titer.

Results

Antibody Response. Antibody titers to GnRH were used to monitor the primary effect of vaccination with Improvac in stimulating an immunological response in pigs. As anticipated, there were no anti-GnRH antibodies detectable (titer < 20) in the serum samples from either control boars or barrows at 2 or 4 wk after secondary treatment (Table 1). Although there was one placebo vaccinated boar in which a very low anti-GnRH titer of 32 was found, this was probably due to nonspecific binding. In contrast, in pigs treated with two doses of Improvac, an immune response against GnRH was detectable in all animals. Although the response was lower in some individual pigs, there were no Improvac-treated pigs in which an immune response was not detected (titer >100). Although there was a slight difference in anti-GnRH titers between the early and late age groups at both the 2- and 4-wk time points, this difference only approached significance at the latter time ($P = 0.56$ and 0.05 for the 2- and 4-wk titers following the second dose, respectively).

Testes Function. In most of the boars treated with either Improvac or placebo vaccine, testosterone was measurable in appreciable (> 2 ng/mL) amounts at the time of second vaccination (Table 1). Furthermore, there were no significant differences in the mean testosterone concentration at the time of second treatment between the different treatment groups ($P = 0.87$).

At the time of the second treatment, 85% of the boars treated with either vaccine or placebo and 2% of barrows had a serum testosterone concentration of > 2 ng/mL, a concentration considered to be biologically significant. Although there was no indication at slaughter, it is likely that the two barrows displaying measurable testosterone were cryptorchids. As expected with a hormone that is secreted episodically,

Table 1. Effect of sex and Improvac vaccine on plasma anti-GnRH titers and testosterone concentrations

	Early[a]				Late[b]			
Item	Placebo	Improvac	Barrow	*P*-value	Placebo	Improvac	Barrow	*P*-value
Plasma anti-GnRH titers[c]								
Secondary dose + 2 wk	10^{x}	$1{,}208^{y}$	10^{x}	< 0.001	10^{x}	$1{,}126^{y}$	10^{x}	< 0.001
	(10)	(1,138)	(10)		(10)	(1,076)	(10)	
	[10–10]	[431–3,500]	[10–10]		[10–32]	[248–3,500]	[10–10]	
Secondary dose + 4 wk	10^{x}	613^{y}	10^{x}	< 0.001	10^{x}	487^{y}	10^{x}	< 0.001
	(10)	(654)	(10)		(10)	(478)	(10)	
	[10–10]	[171–3,500]	[10–10]		[10–10]	[103–1,532]	[10–10]	
Plasma testosterone, ng/mL[d]								
Secondary dose	$13.7^{x} \pm 13.5$	$12.7^{x} \pm 12.3$	$0.29^{y} \pm 31$	< 0.001	$6.61^{x} \pm 5.28$	$8.27^{x} \pm 7.94$	$0.33^{y} \pm 0.56$	< 0.001
	(7.60)	(9.30)	(0.25)		(6.00)	(5.60)	(0.25)	
Secondary dose + 2 wk	$8.52^{x} \pm 7.00$	$0.51^{y} \pm 0.92$	$0.32^{y} \pm 0.46$	< 0.001	$7.03^{x} \pm 6.36$	$0.54^{y} \pm 0.83$	$0.31^{y} \pm 0.40$	< 0.001
	(7.80)	(0.25)	(0.25)		(5.90)	(0.25)	(0.25)	
Secondary dose + 4 wk	$10.5^{x} \pm 6.8$	$1.16^{y} \pm 3.57$	$0.28^{y} \pm 0.21$	< 0.001	$8.26^{x} \pm 6.18$	$0.62^{y} \pm 1.21$	$0.27^{y} \pm 0.17$	< 0.001
	(9.00)	(0.25)	(0.25)		(7.00)	(0.25)	(0.25)	

[a]Pigs received primary vaccination and secondary vaccination and were slaughtered at 15, 19, or 23 wk of age.

[b]Pigs received primary vaccination and secondary vaccination and were slaughtered at 18, 22, or 26 wk of age.

[c]Examination of residuals showed that the assumptions of analysis of variance did not hold. Thus, these data have been analyzed using the Kruskal-Wallis nonparametric analysis of variance and Mann-Whitney U-test to test differences among groups. Values with different superscripts within an age group are different at the 1% level. Data are presented as geometric mean with the median value in parentheses and the range in square brackets.

[d]Examination of residuals showed that the assumptions of analysis of variance did not hold. Thus, these data have been analyzed using the Kruskal-Wallis nonparametric analysis of variance and Mann-Whitney U-test to test differences between groups. Values with different superscripts within an age group are different at the 1% level. Data are presented as mean ± SD with the median value in parentheses.

Table 2. Effect of sex and Improvac vaccine on testes width and testes and bulbo-urethral gland weight and length

	Early[a]				Late[b]			
Item	Placebo	Improvac	$LSD_{0.01}$	*P*-value	Placebo	Improvac	$LSD_{0.01}$	*P*-value
Testes width, mm								
Primary dose	68.0	67.6	3.4	0.80	89.4	87.2	5.6	0.29
Secondary dose	100.6	98.7	5.0	0.32	109.3	110.6	5.9	0.56
Secondary dose + 2 wk	112.3	94.8	4.9	< 0.001	108.5	99.3	4.5	< 0.001
Secondary dose + 4 wk	124.0	93.9	5.4	< 0.001	133.0	102.6	5.9	< 0.001
Change after secondary dose	23.1	–4.8	5.5	< 0.001	23.7	–8.3	5.9	< 0.001
Testes weight, g[c]	421.6	182.6	44.0	< 0.001	509.6	254.4	49.8	< 0.001
Bulbo-urethral gland weight, g[c]	137.4	52.9	20.6	< 0.001	148.0	75.1	26.2	< 0.001
Bulbo-urethral gland length, mm[c]	120.5	86.5	15.4	< 0.001	117.2	93.8	10.7	< 0.001

[a]Pigs received primary treatment and secondary treatment, and were slaughtered at 15, 19, or 23 wk of age.
[b]Pigs received primary treatment and secondary treatment, and were slaughtered at 18, 22, or 26 wk of age.
[c]At slaughter.

some boars had low (< 2 ng/mL) serum testosterone at the time of the second vaccination. Within 2 wk of the second dose of Improvac there was a highly significant reduction in testosterone ($P < 0.001$) in the treated boars such that only 6% of these animals had testosterone concentrations above 2 ng/mL. The effect of the GnRH vaccine on plasma testosterone was maintained until at least 4 wk after the second dose of vaccine, when only 8% of vaccinated boars had testosterone concentrations above 2 ng/mL (Table 1).

There was no significant difference in the mean testes width between the two treatments at the time of either the primary or second vaccination (Table 2). However, within 2 wk of the secondary vaccination testicular growth, as assessed by testes width, had ceased in the treated boars. The suppression in testicular growth was maintained for at least 4 wk after the secondary vaccination (Table 2). For example, over the 4 wk after the secondary vaccination, testes width increased by almost 1 mm/d (+23.4 mm) in the control boars, whereas testes width decreased in the Improvac-treated boars (–6.5 mm). The temporal response in testes width was confirmed by the approximately 50% decrease in testes weight and bulbo-urethral gland weight and length at slaughter in the vaccinated boars (Table 2).

Growth Performance. At 15 wk of age, the barrows were heavier ($P < 0.001$) than the boars, although the relative difference in BW between boars and barrows decreased with age. For both the vaccine and placebo groups, commencing treatment at either 15 or 18 wk of age, there was no difference in the mean live weight at the start of the study, or at the time of the secondary dose (Table 3). In the Improvac-treated pigs, the reduction in testosterone following the second vaccination did not have a detrimental effect on growth performance. Indeed, there was evidence that the Improvac-treated pigs had higher weight gain following the second dose (Table 3). Within the younger pigs, Improvac-treated boars grew 10 and 7% faster than the control boars and barrows, respectively, over the last 4 wk following the second dose of Improvac ($P = 0.05$). However, in the older animals the ADG of Improvac-treated boars was 30 and 32% greater than that of control boars and barrows, respectively ($P < 0.001$), over the last 4 wk. The increased growth of the Improvac-treated boars relative to the control boars seems to be due to an increase in feed intake rather to than any change in feed conversion ratio. For example, feed intake was 19 and 15% higher ($P = 0.006$) in the younger barrows and the boars treated with the Improvac, respectively, than in the contemporary control boars (Table 3). For the older pigs, feed intake was 10 and 16% higher in the barrows and boars treated with the Improvac, respectively, than in the contemporary boars ($P = 0.10$). In both age groups, the feed conversion ratio of control and Improvac-treated boars was similar and less than that of the barrows ($P < 0.04$).

In the younger pigs at slaughter, there was no difference ($P > 0.10$) in backfat thickness at the P2 position between the Improvac-vaccinated and placebo-treated boars (Table 3). As expected, barrows were markedly fatter ($P < 0.01$) than either vaccinated or unvaccinated groups of boars. In the older pigs, there were differences in backfat thickness between all three groups ($P < 0.001$); the barrows were the fattest, Improvac-treated boars were intermediate, and the placebo group had the lowest backfat depth. For both slaughter ages, the barrows had heavier carcasses than the placebo-treated boars ($P < 0.05$) and the carcasses from the Improvac-treated boars were intermediate. Dressing percentage was greater ($P < 0.01$) in barrows than in either placebo or vaccinated boars. For the older pigs, dressing percentage of the Improvac-treated boars was lower ($P < 0.01$) than in the control boars, probably because of the greater feed intake and gut fill in the former group.

Boar Taint. Data on levels of the boar taint compounds androstenone and skatole in subcutaneous fat are provided in Table 4. Placebo-treated boars had fat androstenone levels almost eight times greater ($P < 0.001$) than those of the Improvac-treated boars, which were not different ($P > 0.10$) from the barrows (Table 4). The GnRH-vaccinated boars also had a lower pro-

Table 3. Effect of sex and Improvac vaccine on growth performance

	Early[a]					Late[b]				
Item	Placebo	Improvac	Barrow	$SE_{0.01}$	*P*-value	Placebo	Improvac	Barrow	$SE_{0.01}$	*P*-value
Live weight, kg										
15 wk	52.4	52.7	56.6	2.9	< 0.001	52.9	52.6	56.6	2.8	< 0.001
Secondary dose	74.1	74.0	77.3	3.7	0.032	89.3	88.8	93.4	5.8	0.086
Slaughter	96.2	98.3	99.9	4.7	0.125	113.3	120.7	117.1	6.4	0.013
Average daily gain, g/d[c]	786	868	809	90.0	0.051	858	1119	847	98.5	< 0.001
Feed intake, kg/d[c]	2.44	2.81	2.91	0.35	0.006	2.79	3.40	3.13	0.63	0.097
Feed conversion ratio, g/g[c]	3.03	3.05	3.39	0.38	0.022	3.30	3.10	3.73	0.66	0.035
P2 backfat, mm	11.1	11.9	14.4	1.51	< 0.001	12.6	15.1	17.1	2.01	< 0.001
Hot carcass weight, kg	72.9	74.4	77.1	3.9	0.024	88.6	92.7	93.0	5.4	0.063
Dressing, g/kg[d]	$758^{x} \pm 30.6$ (765)	$757^{x} \pm 22.7$ (758)	$771^{y} \pm 23.9$ (775)		< 0.01	$781^{x} \pm 27.2$ (778)	$768^{y} \pm 17.2$ (768)	$793^{z} \pm 16.5$ (796)		< 0.01

[a]Pigs received primary treatment and secondary treatment, and were slaughtered at 15, 19, or 23 wk of age.
[b]Pigs received primary treatment and secondary treatment, and were slaughtered at 18, 22, or 26 wk of age.
[c]Determined over the last 4 wk prior to slaughter.
[d]Examination of residuals showed that the assumptions of analysis of variance did not hold. Thus, these data have been analyzed using the Kruskal-Wallis nonparametric analysis of variance and Mann-Whitney U-test to test differences between groups. Values with different superscripts within an age group are different at the 1% level. Data are presented as mean ± SD with the median value in parentheses.

Table 4. Effect of sex and Improvac vaccine on fat androstenone and skatole concentrations at slaughter

	Early[a]				Late[b]			
Item	Placebo	Improvac	Barrow	*P*-value	Placebo	Improvac	Barrow	*P*-value
Fat androstenone, μg/g[c]	$1.21^{x} \pm 799$ (1.06)	$0.160^{y} \pm 0.195$ (0.100)	$0.106^{y} \pm 0.041$ (0.100)	< 0.001	$1.05^{x} \pm 0.736$ (0.972)	$0.126^{y} \pm 0.072$ (0.100)	$0.103^{y} \pm 0.022$ (0.100)	< 0.001
Fat skatole, μ/g[c]	0.133 ± 113 (0.090)	$0.068^{y} \pm 0.039$ (0.057)	$0.048^{y} \pm 0.020$ (0.043)	< 0.001	$0.095^{x} \pm 0.074$ (0.080)	$0.056^{y} \pm 0.020$ (0.052)	$0.046^{y} \pm 0.025$ (0.042)	< 0.001

[a]Pigs received primary treatment and secondary treatment, and were slaughtered at 15, 19, or 23 wk of age.
[b]Pigs received primary treatment and secondary treatment, and were slaughtered at 18, 22, or 26 wk of age.
[c]Examination of residuals showed that the assumptions of analysis of variance did not hold. Thus, these data have been analyzed using the Kruskal-Wallis nonparametric analysis of variance and Mann-Whitney U-test to test differences between groups. Values with different superscripts within an age group are different at the 1% level. Data are presented as mean ± SD with the median value in parentheses.

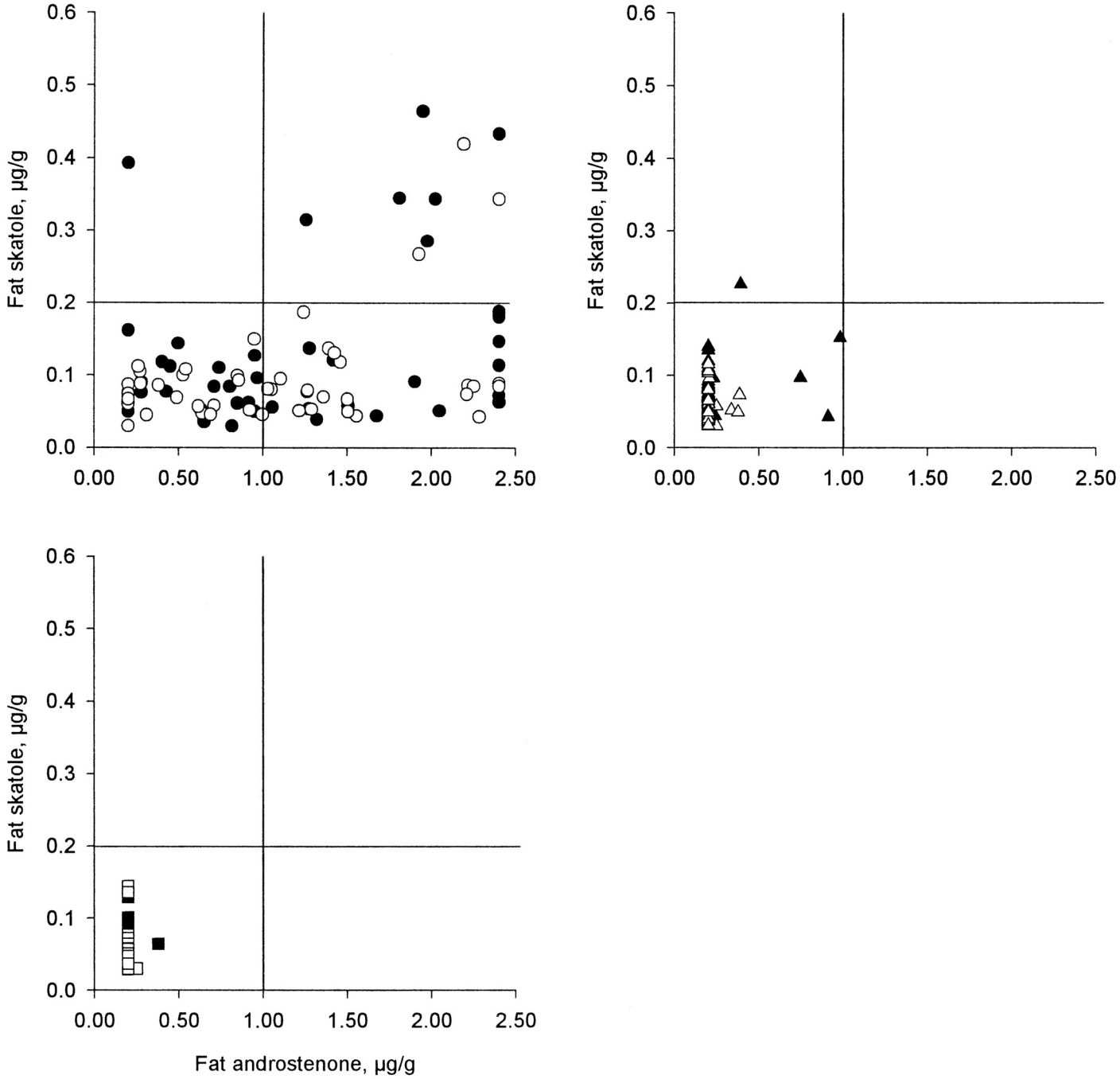

Figure 1. Relationship between fat concentration of skatole and androstenone for placebo-treated boars, (upper left panel, ○ and ● for pigs slaughtered at 23 and 26 wk), Improvac-treated boars (upper right panel, △ and ▲ for pigs slaughtered at either 23 or 26 wk of age, or barrows (lower left panel) slaughtered at either 23 or 26 wk of age. Gridlines indicate upper thresholds for consumer detection of each of the compounds. Individual pigs that lie in the upper right quadrant are likely to be detected as highly tainted by most people.

portion of pigs exceeding an intermediate androstenone threshold of 0.5 μg/kg fat in both age groups, compared with placebo controls (Fisher's exact probability test $P < 0.001$, Figure 1). When pooled across age groups, 24% of control boars had fat androstenone concentrations between 0.5 and 1.0 μg/g and a further 49% had fat androstenone concentrations greater than 1.0 μg/g. In contrast, only 3% of the Improvac-treated boars had intermediate fat androstenone concentrations of between 0.5 and 1.0 μg/g. The remaining vaccinated boars had fat androstenone concentrations well below 0.5 μg/g, and most were below the detection limit. All barrows had fat androstenone concentrations below the lower threshold of 0.5 μg/g.

Placebo-vaccinated boars had fat skatole levels almost twice as high as those of the Improvac-treated

boars ($P < 0.001$), which were not different ($P > 0.10$) from the barrows (Table 4). When pooled across the age groups, 11% of the control boars had fat skatole concentrations above 0.20 μg/g (Figure 1). In contrast, only 1% of the Improvac-treated boars had fat skatole concentrations above 0.20 μg/g, and most Improvac-treated boars had skatole concentrations well below 0.20 μg/g. All barrows had fat skatole concentrations below the lower threshold of 0.20 μg/g.

Figure 1 illustrates the skatole concentrations in fat plotted against the concentrations of androstenone for all three groups. As can be seen, 9.7% of control boars had high concentrations of both androstenone (> 1.0 μg/g) and skatole (> 0.20 μg/g). In comparison, none of the Improvac-treated pigs or the barrows had high concentrations of either androstenone or skatole.

Carcass Lesions and Site Reactions. Twenty-six of the 30 pigs exhibiting wounds attributable to fighting were found to be from the placebo group, and the remainder were Improvac-treated boars ($P < 0.001$). No barrows exhibited fighting scars. The incidence of fighting in Improvac-treated boars and barrows was not different ($P > 0.10$).

Subcutaneous administration of either vaccine or placebo vaccine was generally well tolerated, and the majority of boars showed no reaction following treatment and none visible at slaughter (Table 5). There was no difference in the number of pigs with any detectable reactions following either Improvac or placebo vaccination at either age ($P = 0.24$ and 0.09 for the Improvac and placebo following primary and secondary doses, respectively). There was a modest effect of age on the incidence of site reactions following vaccination. Following the primary dose the incidence of reactions was 26% in the older pigs and only 13% in the younger pigs ($P = 0.03$), whereas after the secondary vaccination 21 and 8% of the younger and older pigs, respectively, exhibited some reaction, $P = 0.02$). It should be emphasized that of the small number of detectable site reactions scoring 1, most of these (~ 70%) were detectable only upon close physical inspection of the injection site. In addition, many of the site reactions scoring 2 were only detectable when the pig moved its head into a certain position. Finally, many of the pigs that exhibited site reactions did so at only one time point.

Discussion

The major outcome from this study was an unequivocal demonstration of the efficacy of Improvac in preventing boar taint, by reducing both androstenone and skatole below threshold levels above which taint may be detected by consumer panels in 100% of vaccinated pigs. Indeed, Improvac treatment of boars was successful in reducing the average androstenone and skatole concentrations to levels similar to those observed in barrows. Although 10% of the placebo-treated boars had high concentrations of both androstenone and skatole, none of the Improvac-treated boars or barrows had high concentrations of either androstenone or skatole. Although there is some debate regarding the actual threshold concentrations of androstenone and skatole that are detectable as taint, the values used here of 1.0 and 0.2 μg/g fat for androstenone and skatole, respectively, are commonly accepted for untrained consumers (Desmoulin and Bonneau, 1982; Bonneau et al., 1992; Bonneau, 1998). Against these thresholds, Improvac was 99 and 100% effective in suppressing skatole and androstenone, respectively. A large multisite European study has demonstrated that both androstenone and skatole have negative effects on consumer perception of pork quality, although there are

Table 5. Site reaction scores in the 28 d following administration of either dose of Improvac or placebo treatment[a]

	Reaction score[b]		
Age group and treatment	0	1	≥ 2
	Primary dose		
Early[c]			
Placebo	42 (88%)	4 (8%)	2 (4%)
Improvac	42 (86%)	2 (4%)	5 (10%)
Late[d]			
Placebo	39 (80%)	8 (16%)	2 (4%)
Improvac	32 (68%)	9 (19%)	6 (13%)
	Secondary dose		
Early[c]			
Placebo	34 (72%)	9 (19%)	4 (9%)
Improvac	42 (86%)	5 (10%)	2 (4%)
Late[d]			
Placebo	44 (90%)	5 (10%)	0 (0%)
Improvac	43 (94%)	2 (4%)	1 (2%)
	Slaughter[e]		
Early[c]			
Placebo	43 (91%)	4 (9%)	0 (0%)
Improvac	47 (96%)	2 (4%)	0 (0%)
Late[d]			
Placebo	46 (94%)	3 (6%)	0 (0%)
Impovac	43 (93%)	3 (7%)	0 (0%)

[a]Number of pigs exhibiting site reactions at any time over the 4 wk after each vaccination. Reaction scores were assessed at weekly intervals.

[b]Data presented as number of pigs presenting with a reaction score (percentages in parentheses).

[c]Pigs received primary treatment and secondary treatment, and were slaughtered at 15, 19, or 23 wk of age.

[d]Pigs received primary treatment and secondary treatment, and were slaughtered at 18, 22, or 26 wk of age.

[e]Pigs at slaughter presenting with either a primary or secondary treatment score (percentages in parentheses).

clearly differences between men and women and people from different regions in levels of each compound that cause dissatisfaction (Bonneau et al. 2000a,b; Dijksterhuis et al., 2000; Mathews et al., 2000). Although the authors stated that it was not possible to determine threshold values, they presented regression equations to predict consumer dissatisfaction (Mathews et al., 2000). Overall, 21.5 and 32.5% and 18.5 and 26.0% of consumers were dissatisfied with the flavor and odor of boars and gilts, respectively (Bonneau et al., 2000b). Thus, 6.5% more consumers would be dissatisfied with the odor of pork from intact males than with that of pork from gilts. The corresponding difference for flavor would be 3%. Their model predicts that the adoption of threshold values of 1.0 and 0.20 μg/g fat for androstenone and skatole, respectively, would essentially halve the differences in dissatisfaction (Bonneau et al., 2000b). However, due to methodological limitations associated with the use of reheated meat for the consumer panels, the panels may have underestimated consumer reaction to pork from intact males.

Taint in meat from boars with high concentrations of both androstenone (> 1.0 μg/g) and skatole (> 0.2 μg/g) is likely to be commonly detected by many consumers, particularly those consumers highly sensitive to androstenone (Bonneau et al., 1992; Weiler et al., 2000). Meat that is high in only one will only be detected by those individuals who are sensitive to that particular compound. However, the presence of one compound may enhance the detection of the other compound. Skatole and androstenone may have synergistic effects (Dijksterhuis et al., 2000); unpleasant odors associated with androstenone can be intensified when high skatole concentrations are found simultaneously (Bonneau, 1982; Annor-Frempong et al. 1997). In the current study, there were no Improvac-treated pigs that had high concentrations of both androstenone and skatole. The few GnRH-vaccinated pigs that had intermediate concentrations of either androstenone or skatole at slaughter did not have significant levels of the other compound.

Studies of meat from vaccinated pigs by sensory panels support the conclusions reached on the basis of reduction in skatole and androstenone concentrations. In an earlier study, vaccination with developmental batches of the GnRH vaccine not only reduced androstenone and skatole, but also improved the sensory characteristics of the meat (McCauley et al., 1997). In terms of boar odor and boar flavor, meat from vaccinated boars was shown to be indistinguishable from meat from female pigs. Meat from the females and vaccinates was judged to be less odorous and more acceptable than meat from unvaccinated boars (McCauley et al., 1997).

In most boars, testosterone was measurable in appreciable amounts (> 2 ng/mL) at the time of second vaccination, either at 19 or 22 wk of age. Indeed, even in some individual pigs as light as 55 kg, active steroidogenesis, as indicated by circulating testosterone, was occurring at the time of second vaccination. Pigs with the steroidogenic capacity to produce high concentrations of testosterone also have the potential to produce androstenone, and hence to have detectable levels of taint in the carcass. A survey of Australian and New Zealand boars revealed high concentrations of both androstenone and skatole in boars as light as 85 kg BW (Hennessy et al., 1997). Therefore, although slaughtering boars at lower weights may reduce the incidence of boar taint, it will not guarantee meat free from boar taint.

The primary dose of the GnRH vaccine seemed to have had no physiological effect on testes function as assessed by the testes size and serum testosterone concentrations at the time of the second dose. However, within 2 wk of administration of the secondary dose of Improvac, testes growth and secretion of testosterone had been suppressed. At slaughter, both testes and bulbo-urethral weights were approximately 50% lighter in the Improvac-treated pigs. These observations clearly indicate the efficacy of the vaccine and simultaneously provide a possible means of monitoring compliance.

When treatments such as Improvac have been used to control boar taint, on-line screening for testes and(or) bulbo-urethral gland size may be a very suitable method to detect those few individuals in which the vaccination may not have been fully effective, or as a convenient method of checking producer compliance. For example, in the present study, two of the three GnRH-vaccinated boars with intermediate fat androstenone (0.5 to 1.0 μg/g) concentration and the individual treated pig with a moderate fat skatole concentration were among those individuals that had the larger testes and(or) bulbo-urethral glands. A suitable cut-off for testes size indicative of effective vaccination might be 350 and 400 g for the 23- and 26-wk-old pigs, respectively, of this genotype raised under these particular conditions (Figure 2). In this scenario, if 23 and 26 wk old boars are certified as having been vaccinated with Improvac and have paired trimmed testes weight less than about 350 and 400 g, respectively, the processor can be confident that they will be free of taint. Of course, other cut-offs might be applicable under different circumstances.

One of the aims of this study was to ensure that growth performance was not compromised by GnRH vaccination treatment, as is the case with barrows that exhibit inferior feed conversion efficiency compared with intact boars (Campbell and Taverner, 1988; Dunshea et al., 1993b). The growth rate of the Improvac-treated boars was improved over that of the placebo-treated boars, and the feed conversion efficiency was unchanged. A recent study comparing immunocastrates with intact boars showed that immunocastrates and intact boars performed equally and both had a feed conversion efficiency about 10 to 12% better and a 3 to 4% higher lean meat yield than barrows (Bonneau et al., 1994). Using three injections spaced

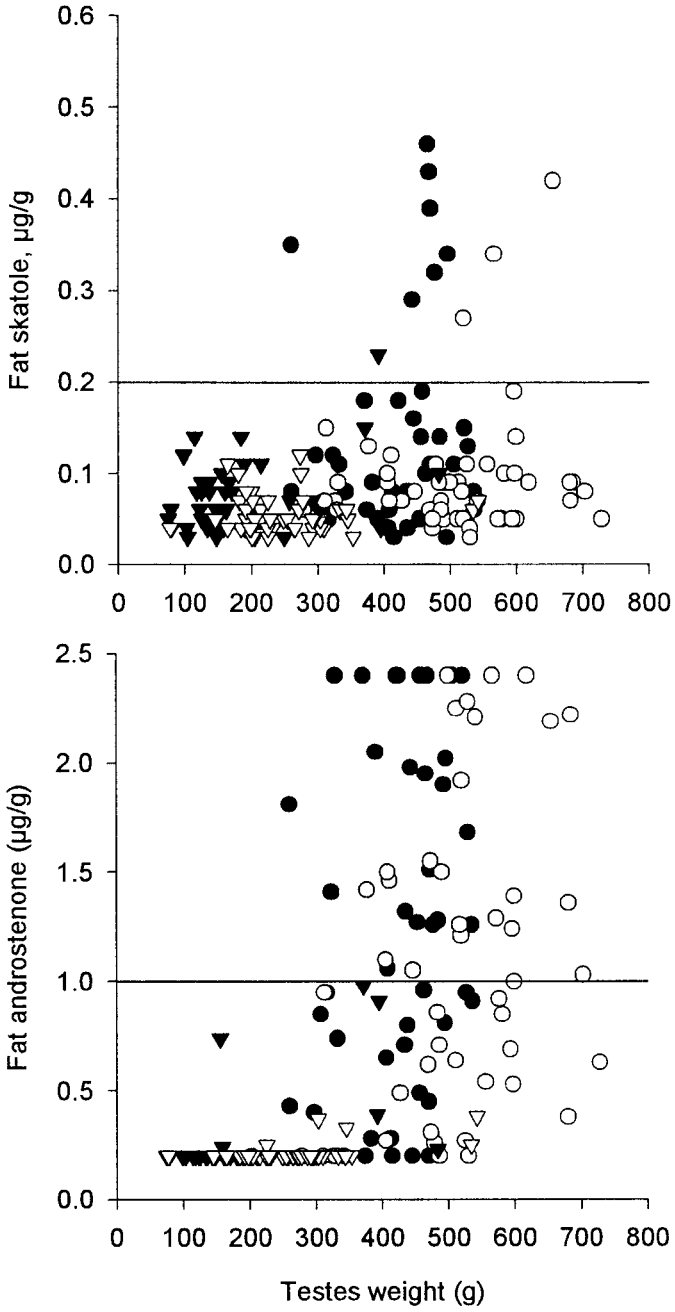

Figure 2. Relationship between fat skatole (upper panel) and fat androstenone (lower panel) and testes weight in placebo-treated boars (○ and ● for pigs slaughtered at 23 and 26 wk) and Improvac-treated boars (▽ and ▼ for pigs slaughtered at 23 and 26 wk of age). Gridlines indicate upper thresholds for consumer detection of each of the compounds.

at 4-wk intervals and commencing at 8 wk of age, Dunshea et al. (1993a) also demonstrated that vaccination with a GnRH preparation was highly effective in stopping testicular function and growth. In that study, vaccination had no effect on average daily gain but did result in a large increase in backfat thickness. In the present study, the better growth performance following the secondary vaccination may be related to a decrease in aggressive and sexual behavior as a consequence of suppression of testicular function. At around 19 to 22 wk of age when the secondary vaccination was given, pigs are well advanced in puberty and normally display an increasing amount of sexual behaviors and related aggression. Stookey and Gonyou (1994) reported an increase in the level of fighting and a corresponding decrease in growth performance in finishing gilts and barrows after social regrouping. It is likely that these effects would be exacerbated in boars. In the present study, the decrease in fighting lesions exhibited after transport and lairage in Improvac-treated boars is consistent with a reduction in aggressive behaviors in these animals.

Castrated pigs consume more feed than boars (Campbell and Taverner, 1988; Dunshea et al., 1993b), and this is probably related to the low concentrations of testosterone in barrows. The wild boar, which exhibits wide seasonal fluctuations in testosterone, stops eating when testosterone concentrations are at their peak (Weiler et al., 1996). In the domestic boar, which exhibits less marked seasonal variation in both plasma testosterone and feed intake, there is still a strong negative correlation ($P < 0.001$) between testosterone and feed intake (Weiler et al., 1996). Therefore, the reduction in testosterone may account for the increased feed intake that occurs after immunization against GnRH. Whether the reduced feed intake is a direct effect of testosterone on satiety is unknown. For example, it may be possible that the increase in feed intake occurs because the vaccinated boars are no longer involved in sexual or aggressive activities that detract from time spent eating. It is also possible that the lower aggression and sexual behavior allows energy to be directed toward carcass growth rather than into unproductive activities. Although there is little change in feed conversion efficiency it may be that the energy saved by not fighting is negated by the small inefficiency associated with the slight increase in fat deposition in Improvac-treated boars. Finnerty et al. (1996) have shown that bulls vaccinated with a similar GnRH vaccine displayed less sexual activity and lower aggression compared with control bulls when observed at pasture. Thus, the improved growth performance following GnRH vaccination results partially from reduced sexual and aggressive activities and less stress over the last weeks preceding slaughter.

Furthermore, it is quite likely that the reduction in stress and fighting in the 24-h preslaughter in the Improvac-treated pigs may result in improved meat quality. Fighting and aggression, due to mixing of unfamiliar boars before slaughter, is related to a reduction in meat quality due to an increase in the incidence and severity of dry, firm, and dark pork (Sather et al., 1995; D'Souza et al. 1999). Fighting-induced physical activity in response to aggressive interactions in pigs depletes muscle glycogen, thus affecting the ultimate pH and meat quality (Fernandez et al., 1994; D'Souza et al., 1999). Alternatively, if the lairage and transport

times are short, stress around slaughter may increase the incidence of pale, soft, exudative pork (D'Souza et al., 1998). In either circumstance, it is possible that boars vaccinated against GnRH may have better meat quality than boars, although this was not assessed in the present study.

One of the issues faced during the development of an immunocastration vaccine is how to present the antigen in a manner that will create sufficient antibody response without using an adjuvant that will cause considerable site reactions and tissue damage. Early studies with oil-based vaccines resulted in high antibody titers, but these vaccines were associated with an unacceptable incidence of site reactions. Subcutaneous injection of oil-based vaccines, particularly those containing mineral oils, can cause site lesions in pigs (Hall et al., 1989; Straw et al., 1985, 1990). Although this may not be a great problem in pigs slaughtered many months after vaccination (Oishi et al., 1997), it is an important consideration when pigs are being slaughtered a short period of time after vaccination, such as is the case for the use of an immunocastration protocol that makes optimum use of the male characteristics of boars. The aqueous proprietary adjuvant system that has been developed for Improvac does not contain oil and causes very little irritation to the pig. The injection sites on the vaccinated pigs were assessed weekly for signs of tissue reaction and there was a very low incidence of any visible or palpable reactions. In addition, the injection sites were carefully inspected and palpated to detect any residual reaction at slaughter. There were no visible site reactions in any pigs at slaughter, whereas only 12 out of 192 vaccinated pigs had a reaction that could be detected by palpation. Approximately 400 doses of vaccine (200 each of active and placebo) were administered during this study. Of these vaccinations, there was only one case of an abscess or serious site reaction that may have been attributable to the vaccine. This pig was detected with a visible swelling at 21 d after the primary vaccination. At slaughter it presented fully healed as a scar only.

Implications

The major outcome from this study was a clear demonstration of the efficacy of Improvac in preventing boar taint, by reducing both androstenone and skatole below their accepted thresholds in 100% of vaccinated pigs. Vaccination with Improvac also increased growth rate and feed intake, although backfat was also increased slightly. The incidence of fighting lesions at slaughter was also decreased markedly in pigs vaccinated against GnRH. The use of Improvac will allow full use of the efficient growth performance of boars while maintaining product quality.

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